Response of Nitrogen Fixing Non-leguminous *Alnus nepalensis* D. Don and Leguminous *Glycine max* (L.) Merr. to Selected Invasive Species

A Dissertation Submitted for the Partial Fulfillment of Master's Degree in Botany

Submitted by

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DECLARATION

I Sangita Pachhai, student of M.Sc. Botany, Central Department of Botany, TU, Kritipur, hereby declare that the research work entitled "**Response of Nitrogen Fixing Non-leguminous *Alnus nepalensis* D. Don and Leguminous *Glycine max* (L.) Merr. to Selected Invasive Species "** submitted by myself at Central Department of Botany, Institute of Science and Technology, Tribhuvan University for **Partial Fulfilment of Master's Degree in Botany,** is a record of bonded work carried out by me under the supervision of **Asst. Prof. Dr. LB Thapa and Assoc. Prof. Dr. Chandra Prasad Pokhrel,** Central Department of Botany, Tribhuvan University, Kritipur, Kathmandu, Nepal.

I further declare that the work reported in this research work has not been submitted and will not be submitted for the award of any other degree in this or any other institutions.

………………………………

Sangita Pachhai

April, 2019

RECOMMENDATION

This is to certify that **Ms. Sangita Pachhai** has successfully completed her dissertation work entitled **"Response of Nitrogen Fixing Non-leguminous *Alnus nepalensis* D. Don and Leguminous *Glycine max* (L.) Merr. to Selected Invasive Species"** under our supervision. This work is based on the research work completed by the herself and the result of this work have not yet been submitted for any other academic degree. We, therefore, recommend her work for approval and acceptance for the partial fulfillment of Master's Degree in Botany from Tribhuvan University.

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LETTER OF APPROVAL

This dissertation work entitled **"Response of Nitrogen Fixing Non-leguminous *Alnus nepalensis* D. Don and Leguminous *Glycine max* (L.) Merr. to Selected Invasive Species"** submitted to the Central Department of Botany, Tribhuvan University by Ms. Sangita Pachhai has been accepted for the partial fulfillment of Master's of Science in Botany.

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Sangita Pachhai

ABSTRACT

Two of the invasive alien species viz. *Ageratina adenophora* and *Parthenium hysterophorus* are the most problematic invasive alien species in various ecosystems of Nepal including forests and agroecosystems. Their impacts on wild native plant species and domesticated crop species is an urgent task to be documented to understand their mechanism of invasion and developing controlling strategies. These two invasive species have been selected in this study to explore their effects on wild non-leguminous nitrogen fixing tree *Alnus nepalensis* and a leguminous crop plant *Glycine max.* Their effect on growth and development and root nodulation of both the nitrogen fixing selected test plants was measured. Soil pH and nitrogen content after treatment of invasive species litter and extracts was also measured. For this, pot experiment was conducted at Central Department of Botany, Tribhuvan University, Kathmandu, Nepal. A peculiar interaction among the invasive species extracts, litter, invaded soil by *A. adenophora* and non-invaded soil is seen in the experiment. Addition of *A. adenophora* leaf extract on non-invaded soil showed inhibition to root shoot length and biomass as well as leaf and nodule number in *A. nepalensis.* The interaction of fresh leaf extract with invaded soil did not inhibit these parameters. Additionally, the invaded soil was found associated with lower nitrogen content in soil and in response the nodule number was found increased in *A. nepalensis.* In case of *G. max,* only leaf number and shoot biomass was reduced by both invasive species fresh leaf extract. Root nodules in *G. max* was increased by *Ageratina adenophora* leaf extract but the nodules were inhibited by *P. hysterophorous.* Hence, the fresh leaf extract of both the invasive species are detrimental for leguminous and non-leguminous nitrogen fixing plants but *A. adenophora* is associated with beneficial interaction for the formation root nodules. It is recommended that these invasive species should be controlled in native and agroecosystem as they can have detrimental effect on native and crop plants. Further studies should be carried out to confirm the interactions shown from this study.

Keywords: Invasive alien species, invaded and non-invaded soil, soil nitrogen, root nodulation

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ABBREVIATIONS

°C	Centigrade
cm	centimeter
Fig	Figure
FLE	Fresh leaf extract
g	gram
IAPS	Invasive alien plant species
LI	litter
L	Litre
IS	Invaded soil
NIS	Non-invaded soil
No.	Number
S.D.	Standard Deviation
S.E.M	Standard Error of Mean
sp.	Species

CHAPTER - I

INTRODUCTION

Invasive alien plant species (IAPS) are known to have detrimental effects on native biodiversity, ecosystem functioning and people's livelihood (Sagoff 2005; Rai *et al.* 2012; Roy *et al.* 2014). Rapid expansion of invasive alien plants and their threats to biodiversity, ecosystems, crop productivity as well as human health have become serious global issues (Pejchar *et al.* 2009; Moles *et al.* 2012). The IAPS are able to competing native plants for resources, space and potential change in above and below ground community (Mack and D'Antonio 2003; Balami *et al.* 2017). They also bring changes in species richness, composition, native species growth and developments, and ultimately replace the native community by establishing their monoculture (Callaway *et al.* 2004, Reinhardt and Callaway 2004).

1.1. Mechanism of invasion

Transport, colonization, establishment and landscape spread may be different steps in IAPS invasion process. There are different hypotheses to explain the mechanism of plant invasiveness and among them some of the most highlighted hypotheses are Phenotypic Plasticity, Disturbance, Empty Niche Hypothesis (ENH), Enemy Release Hypothesis (ERH), Novel Weapon Hypothesis (NWH) and the Evolution of Invasiveness Hypothesis (EIH) (Hierro *et al.* 2005; Holzmueller and Jose 2009).

Phenotypic plasticity of IAPS has been considered as one of the major means by which they can cope with varied environmental factors (Rai 2015). Disturbance, plays a prime role in invasion ecology. Several studies have illustrated key role of disturbances on invasion success of several IAPS (Rai 2015). According to the Empty Niche Hypothesis (ENH), IAPS can utilize resources which have not been utilized by native species and hence empty niche helps alien species to establish and invade into a new environment (Gioria and Osborne 2014).

Enemy Release Hypothesis (ERH) states that the IAPS in an introduced range experiences a smaller number of natural enemies, resulting in its rapid increase in distribution and abundance (Keane and Crawley 2002). The Novel Weapon Hypothesis (NWH) is a very commonly understood hypothesis which states that the IAPS can produce certain novel chemicals that may inhibit native plants and alter soil quality (Callaway and Ridenour 2004). Finally, character modification by IAPS for adapting to new environment and hybridization are important phenomena behind invasion success which is related to the evolution of invasiveness hypothesis (EIH) (Ellstrand and Schierenbeck 2000; Novak 2007).

1.2. Impacts of invasive species

The impacts which can be observed in native species or ecosystem after alien plant invasion are the combined outcome of complex interaction between alien invaders, belowground biota and soil (Gioria and Osborne 2014). Recent studies have shown that some of the invasive species are found associated with accumulation of soil pathogenic microbes that can harm native species and others inhibit growth and development of useful soil microbes or soil flora/fauna (Callaway *et al.* 2004; Mangla *et al.* 2008; Balami *et al.* 2017). Ultimate impact of interaction among invasive species, soil and soil biota creates abnormal soil feedback mechanisms for native species (Inderjit *et al.* 2011, Thapa *et al.* 2016a, 2017).

The IAPS are known to have adverse effect on biodiversity (Pimentel *et al.* 2005). They are considered as the second most important factor after habitat destruction in loss of biodiversity (Lowe *et al.* 2000). Their abilities of producing varied harmful allelochemicals are responsible for altering soil quality (Callaway *et al.* 2001; Hinsinger *et al.* 2005). The allelochemicals reduce native seed germination, seedlings growth and development (Timsina *et al.* 2011; Thapa *et al.* 2017).

Most of the studies concerning plant invasiveness and its impacts focus aboveground higher plant communities but governing role of plant-soil microbial interaction is rarely investigated (Balami *et al.* 2017). Below ground microbial community has very important role to shape the above ground plant communities but linking the effect of belowground community to above ground community is usually given less priorities (Callaway *et al.* 2001). Giving priority to this relationship by linking the role of invasive alien species could be very interesting in biological investigations.

1.2.1. Invasive alien species in Nepal

In Nepal, 25 species are considered as the IAPS (Shrestha *et al.* 2016). The species such as *Ageratina adenophora, Lantana camara, Chromolena odorata and Mikania micrantha* are widely distributing in the forests, grassland, fallow land, road side etc. (Shrestha 2016; Thapa *et al.* 2016). The species such as *Parthenium hysterophorus, Ageratum houstonianum* and *Alternanthera philoxeroides* are distributed in various agroecosystems. One of the harmful invasive species *Eichhornia crassipes* has created severe problem in wetlands. Regarding impacts, these species have created various negative impacts on ecosystems, for example: native and endangered species have been decreased in nature and are displaced from their natural habitats (Timsina *et al.* 2011). The species which are introduced in the agricultural fields have impacted crops plants causing significant amount of economic losses.

1.2.2. *Ageratina adenophora* and *Parthenium hysterophorus*

Among problematic invasive alien species in Nepal *Ageratina adenophora* and *Parthenium hysterophorus* are aggressively growing in different agroecosystems and forests (Shrestha 2016). They are known to have allelopathic effect on many crops and native species (Singh *et al.* 2003; Timsina *et al.* 2011; Inderjit *et al.* 2011; Thapa *et al.* 2017).

Ageratina adenophora, called Crofton weed and Kalo Banmara in Nepali, is native to Mexico was first reported in 1952 in Nepal (Lowe *et al.* 2000; Wang and Wang 2006). It is widespread and forms monoculture stands displacing native species and disrupting ecosystem processes (Shrestha 2016). As *A. adenophora* has invaded forests, crop fields and found around the agro-ecosystem it may have direct or indirect impacts on wild native and crop plants.

It is found associated with native species replacement, altered soil physico-chemical parameter, reduced native species growth and development such as *Schima wallichii* (D.C.) North and *Alnus nepalensis* D. Don. (Thapa *et al.* 2016, 2017). Currently it has heavily distributed in different ecosystems mainly in subtropical forest ecosystems (Tiwari *et al.* 2005). Thapa *et al.* (2016) found that fresh leaf leachates of *A. adenophora*, volatiles from its litter and *A. adenophora* invaded soil have toxic effects on growth and development of native *S. wallichii* and *A. nepalensis.*

Another invasive alien plant is *P. hysterophorus* which is commonly called Parthenium and *Pati Jhar* or *Maobadi Jhar* in Nepali. It is one of the noxious invasive species belong to Asteraceae family, originated from North and South America and West Indies and introduced in Nepal in 1967 (Picman and Picman 1984; Shrestha 2016). This plant is an annual weed found in tropical and subtropical region but nowadays reach to temperate region in Nepal and infesting both fallow and cultivated field (Tiwari *et al.* 2005). It has altered species composition and soil nutrients of grasslands (Timsina *et al.* 2011). It also causes allergic dermatitis to human (Shrestha *et al.* 2015) and the species has invaded crop fields.

1.2.3. Does invasive species affect root nodulation?

An interesting phenomenon associated with *Alnus nepalensis* is that there are root nodules for nitrogen fixation. The nodules host a kind of bacterium called *Frankia* which helps to fix atmospheric nitrogen (Masson-Boivin *et al.* 2009; Franche *et al.* 2009). Due to this reason *Alnus* shows very fast growth and development in wide range of disturbed habitat. It can be noted that *A. adenophora* also invades in the disturbed habitats where *A. nepalensis* grows faster and well. From this it can be expected that there might have competition between native *Alnus* and invasive *Ageratina.*

Parthenium hysterophorus is also known to have allelopathic in nature where water soluble phenolic and sesquiterpene lactones and mainly parthenin are found in root, stem, leaves, inflorescence, pollen and seeds (Pandey 2009; Beltz *et al.* 2008; Rashid *et al.* 2008). Soyabean (*Glycine max*) is one of the leguminous and highly protein containing plant, have biological nitrogen fixing bacteria (*Bradyrhizobium*) in their root which help to enhancing the soil fertility (Zhang *et al.* 1996).

Nitrogen fixing plants have ability to fix nitrogen. There are many nitrogenous plants which aren't depend on soil nitrogen because they have capability to fix atmospheric nitrogen into molecular nitrogen but other non-nitrogen fixing plants are depend on artificial nitrogen sources such as urea. Invasive alien plants are invaded aggressively all over the world; show their negative impact on ecosystems, soil dynamic and others. Such type of impact shown in nitrogen fixing plants but they have nitrogen fixing bacteria in their root. Thus, there is little bit work on invasive plants effect on nitrogen fixing plants. In this thesis two different nitrogen fixing forest plant *Alnus nepalensis* and agricultural plant *Glycine max* were selected.

1.3. Justification

Invasive alien plants have mainly negative effects on growth and development of many native and crop plants. They have been causing great economic loss worldwide in their invaded range. Invasive *A. adenophora* and *P. hysterophorus* are among detrimental invasive species which have been causing severe problems on forest and agricultural lands respectively. It is thought that *A. adenophora* might have effect on root nodulation in non-leguminous tree *A. nepalensis,* and both *A. adenophora* and *P. hysterophorus* as agriculture land invaders might have effect on root nodulation in one of the most important leguminous plant Soybean (*Glycine max*).

Plant-soil and microbial interaction is one of the very complex and important mechanism affecting plant growth and developmental parameters. In *A. nepalensis* the microbes *Frankia* is associated and in *G. max* bacteria *Rhizobium* is associated for nodule formation. It is always significant to know the impact of invasive species in soil, and then in soil microbes associated with nodule formation and finally, in the root nodules and plant growth/development.

Study of effects of invasive species on nodule forming microbes may become a single and extensive part of study. Hence, under treatment of invasive plants how nodules response in terms of nodule numbers and plant growth parameters has been explored in this study. In addition, soil pH and nitrogen were considered as the factors affecting root nodules and are the factors affected by invasive species. Importantly, soil bacteria and mycorrhizae are very important factors that affect plant growth, nodulation and nodule forming bacteria. Enumeration of such microbes has to be carried out to understand interactions among soil characteristics, nodulation, plant growth and development.

1.4. Objective

1.4.1. General objective

Major objective of the study is to investigate the interaction effect of invasive plants and soil microbes to nodulation, growth and development of nitrogen fixing native tree *Alnus nepalensis* and leguminous crop plant *Glycine max*.

1.4.2. Specific objectives

- To identify the effect of selected invasive plants on plant growth and developmental parameters of *G. max* and *A. nepalensis.*
- To determine the effect of selected invasive plants on root nodulation of leguminous *G. max* and non-leguminous *A. nepalensis*.
- To determine the effect of selected invasive plants on soil nitrogen and pH.
- To determine the effect of selected invasive plant on mycorrhizal association.

1.5. Limitation

- Both the test plants *A. nepalensis* and *G. max* were grown in pots and experiments were run in greenhouse of Central Department of Botany.
- The temperature and moisture were not controlled in the greenhouse. The plants were allowed to grow in greenhouse conditions. Temperature and moisture data were recorded daily
- Effect on plant growth was observed in vegetative stage only.

CHAPTER - II

LITERATURE REVIEW

2.1. Impacts of IAPS: a global scenario

Lonsdale (1999) showed that the New World is significantly more invaded than the Old World and the islands are more invaded than mainland sites. He also showed that the degree of invasion increased with latitude, but there was no such relationship for islands.

Mooney and Cleland (2001) explained several examples of invasive species altering the evolutionary pathway of native species by competitive exclusion, niche displacement, hybridization, introgression, predation, and ultimately extinction. They highlighted examples of extinction that are associated with competitive interactions and interactions between invasive and native biota demonstrating how global changes alter community structure.

Ehrenfeld (2003) concluded that the IAPS alter soil nutrient dynamics by differing from native species in biomass and productivity, tissue chemistry, plant morphology, and phenology. They suggested that the IAPS frequently increase biomass and net primary production, increase N availability, alter N fixation rates, and produce litter with higher decomposition rates than co-occurring natives.

Pimentel *et al.* (2005) estimated that approximately 50,000 foreign species have been introduced in USA causing major environmental damages and economic loss. They approximated that about 42% of species are threatened or becoming endangered due to alien plant invasion. Similarly, Didham *et al.* (2005) reviewed about the causes of biodiversity loss due to alien plant invasions.

Reinhart *et al.* (2006) evaluated the effects of invasive *Acer platanoides* and found that the *Acer* trees appear to produce a more mesic environment by modifying the structure and phenology of the forest canopy and by altering the timing of transpirational water loss relative to *P. menziesii*.

Holmes *et al.* (2009) conducted an economic evaluation of forest-invasive species and highlighted the greatest economic impacts of invasive species in forests are due to the loss of nonmarket values. They proposed that new methods for evaluating aggregate economic damages from forest invasive species need to be developed that quantify market and nonmarket impacts at microscales.

Dogra *et al.* (2010) concluded that the plant invasion is a potent threat to the species diversity. They highlighted the importance of understanding the process of invasion and their impact on species diversity in various habitats to preserved indigenous species diversity.

Gallardo *et al.* (2016) stated that the expansion of invasive macrophytes caused the largest decrease in fish abundance, planktonic communities and benthic invertebrates. They proposed a framework that incorporates both direct biotic interactions (predation, competition, grazing) and indirect changes to the water physicochemical conditions mediated by invaders (habitat alteration).

Fu *et al.* (2018) studied the effects of *A. adenophora* on the composition and structure of Yunnan, China. They found that *A. adenophora* alter species and functional diversity due to presence of leaf area, leaf nitrogen concentration and leaf phosphorous concentration compare to native species. Their result suggests that *A. adenophora* causes loss of leaf nitrogen concentration and the invasion also decreases the growth of seedling of native canopy tree.

2.2. Novel weapon hypothesis and allelopathy of IAPS

Callaway and Ridenour (2004) discussed a new theory for invasive success "the novel weapons hypothesis". They proposed that some invaders possess novel biochemical weapons that function as unusually powerful allelopathic agents or as mediators of new plant–soil microbial interactions.

Vivanco *et al.* (2004) identified a chemical – 8-hydroxyquinoline from the root exudates of invasive *Centaurea diffusa*. This chemical was at three times more concentrated in *C. diffusa* invaded North American soils than in that weed's native Eurasian soils and had stronger phytotoxic effects on grass species from North America. They suggested that the Eurasian plants and soil microbes may have evolved natural resistance to 8-hydroxyquinoline while North American plants have not, suggesting a remarkable mechanism by which exotic weeds destroy native communities.

Orr *et al.* (2005) analyzed responses of native tree species to potential allelopathic effects of invasive plant species *Lolium arundinaceam* and *Elaeagnus umbellate* on three common successional tree species: *Acer saccharinum*, *Populus deltoides*, and *Platanus occidentalis*. They had applied aqueous extracts derived from soil, leaf litter, or live leaves to native trees. They found that tall the *Lolium* reduced the probability that seedlings emerged, and minced leaves of *Elaeagnus* reduced the number of days to emergence. They suggested that the allelopathy may be one mechanism underlying the negative impacts on native species.

Stinson *et al.* (2006) presented novel evidence that antifungal phytochemistry of the invasive plant, *Alliaria petiolate.* The species suppresses native plant growth by disrupting mutualistic associations between native canopy tree seedlings and belowground arbuscular mycorrhizal fungi. They elucidated an indirect mechanism by which invasive plants can impact native flora which is helpful to explain how the alien plant successfully invades relatively undisturbed forest habitat.

Niu. *et al.* (2007) tested the physiochemical parameters of soil invaded by *Ageratina adenophora*. They found that *A. adenophora* invasion resulted in reduced actinomycetes and fungal communities in heavily invaded sites as compared to non-invaded sites.

Jordan and Larson (2008) analyzed the invasive properties of *Eurphorbia esula, Bromus inermis* and *Agropyron cristatum*. They found that *Bromus* and *Agropyron,* exhibited significant self-facilitation via soil modification. Both the species have also had significant facilitative effects on other invasiveness via soil modification. Both *Agropyron* and *Euphorbia* consistently suppressed growth of native forbs.

Callaway *et al.* (2008) found that one of North America's most aggressive invaders *Alliaria petiolata* which inhibits mycorrhizal fungal mutualists of North American native plants. They suggested that the antifungal effect was due to specific flavonoid fractions present in *A. petiolata* extracts as novel weapons.

Thorpe *et al.* (2009) conducted experiments in the field in two different years in the native (Romania) and invaded (Montana, USA) ranges of *Centaurea maculosa*, testing the effects of catechin on species that co-occur with *C. maculosa* in both ranges. They found that the catechin reduced the growth of native plant species in Montana in both years but there was no effect of the catechin on plants in Romania.

Kim and Lee (2011) found that *Eupatorium rugosum* is the invasive species that produce higher concentrations of total phenolic compounds than native species compared. Extracts having the phenolic compounds of invasive species reduced radicle and shoot growth of the native species. It was noted that phenolics were just one component of a plant's potential allelopathic arsenal.

2.3. IAPS in Nepal

There are 219 alien species of flowering plants and 64 species of animals that are naturalized in Nepal (Tiwari *et al.* 2005). Tiwari *et al.* (2005) mentions 21 naturalized alien species are the problematic IAPS in Nepal in an assessment of IAPS undertaken in by IUCN Nepal during 2002 to 2003. Shrestha (2016) mentioned additional four naturalized species such as *Ageratum conyzoides, Erigeron karvinskianus, Galinsoga quadriradiata* and *Spermacoce alata* as the invasive categories which are distributing in agro-ecosystems and rangelands.

Siwakoti *et al.* (2007) suggested that *Mikania micrantha* was well established IAPS causing serious problems in forest, grass land, fallow lands, crop land and wet land of tropical parts of eastern and central Nepal. This species was considered as a responsible species in blocking sunlight for other natives.

Baral *et al.* (2010) found that two invasive species (*Ageratum houstonianum* and *Ageratina adenophora*) as the problematic IAPS throughout the Panchase area of Nepal. They illustrated that the invasion was fueled by anthropogenic disturbances such as leaving the agricultural lands, fallow and degradation of habitat. They reported many negative consequences of the invasion in the study sites.

Timilsina *et al.* (2011) observed ecological impact of *Parthenium hysterophorus* in Nepal and found that the *Parthenium* replaced native and non-native species with alteration in soil nutrients (NPK) contents.

Rai *et al.* (2012) examined how rural people in the buffer zone of Chitwan National Park in Nepal perceive the effects of IAPS such as *Mikania micrantha, Lantana camara* and *Chromolaena odorata* and impact on peoples' livelihoods. They found that the farm households are likely to adapt to the invaded environment as they have a history of interacting with invasive plants. They explained about effort of people on control and management of invasive plants.

Bhattarai *et al.* (2014) studied data retrieved from published literatures and herbarium specimens and found that the native plant species and invasive plant species have similar distribution patterns. They also found a clear trend of higher invasive plant richness in regions where native tree species richness is relatively high. Their conclusion was that the invasive plant species richness correlates positively with anthropogenic factors such as human population density and the number of visiting tourists.

Thapa *et al.* (2016a) studied plant communities invaded by *Ageratina adenophora* in Champadevi hill forest in central Nepal. They found that *Schima-Alnus* mixed forest in the area is highly invaded by *A. adenophora.* They found that, although the level of invasion is low, *A. adenophora* has reached on the top of hills where *Quercus* and *Pinus* are the dominated elements. Similarly, Thapa *et al.* (2016b) studied the impact of *Chromolaena odorata* in tropical Sal (*Shorea robusta*) forest of Nepal. They found that the *C. odorata* is highly problematic as it has reduced native species richness, density and recruitment of Sal seedlings in the invaded regions.

Thapa *et al.* (2017) examined the effect of invasive *Ageratina adenophora* leaf litter and its invaded soil on seedling growth and development of native tree *Schima wallichii.* They found that both the invaded soil and the *A. adenophora* litter are harmful for the development of root growth (length and biomass) of native *S. wallichii.* They also found that the lower pH of invaded soil might have negative impacts on seedling growth and development of native species in the invaded sites.

2.4. Impacts of IAPS on soil biotic communities

Marler *et al.* (1999) suggested that arbuscular mycorrhizal fungi enhance the ability of *Centaurea maculosa* to invade native grasslands of western North America.

Reinhart and Callaway (2004) suggested that invasion of *Acers* species is enhanced by soil biota associated with dominant native species. Their results also suggested that the mutualists are relatively more beneficial to invasive *Acers* in their nonnative ranges than in their native ranges.

Batten *et al.* (2006) compared the microbial communities of invasive and native plant rhizospheres in serpentine soils. They found differences in rhizosphere microbial communities among two invasive species (*Centaurea solstitialis* and *Aegilops triuncialis*) and five native species (*Lotus wrangelianus*, *Hemizonia congesta*, *Holocarpha virgata*, *Plantago erecta*, and *Lasthenia californica*). They showed changes in soil microbial community composition induced by plant invasion which is responsible for adverse effect on native plant fitness and/or ecosystem function.

Van der Putten *et al.* (2007) stated that the invasive plants and animals can have major effects on microbial decomposition in soil. They suggested future studies on understanding, predicting and counteracting consequences of IAPS to know how soil microbes interact, how they are influenced by higher trophic level organisms and how their combined effects are influencing the composition and functioning of ecosystems.

Wolfe *et al.* (2008) suggested that the invasive *Alliaria petiolata* inhibits the growth of Ectomycorrhizal fungi in forests of its introduced range. This change may influence tree seedling establishment and biogeochemical cycling. Mangla *et al.* (2008) showed that the impacts of *Chromolaena* are due to the exacerbation of biotic interactions among native plants and native soil biota.

Van der Putten (2010) explained plant–soil feedback interactions in IAPS invasion. He proposed that the interaction in the invaded range are neutral to positive, whereas native plants predominantly suffer from negative feedback effects. In additions, he suggests that the exotic plants can manipulate local soil biota by enhancing pathogen levels or disrupting communities of root symbionts. Lorenzo *et al.* (2010) investigated the effect of *Acacia dealbata* invasion on the structure of soil fungi and bacteria communities in Northwest Spain pine forest, shrubland and grassland. They showed a clear effect of the invasion on the overall structure of microorganism communities. There were significant differences in soil microorganism's richness and diversity between invaded and not invaded soils. Grassland invaded by *A. dealbata* lead to a significant increase of bacterial richness but a significant reduction in fungal richness and diversity.

Balami *et al.* (2017) compared species richness of soil fungi in *Ageratina adenophora* invaded and non-invaded soils. They found lower richness of soil fungi in the *A. adenophora* invaded soil compared to the uninvaded soil. The occurrence frequency of particular fungi was different for those two soil conditions. Moreover, their study suggests that the *A. adenophora* also alters soil fungi species composition as the invasion replaces saprophytic fungi and accumulates the pathogenic fungi. They concluded that that the invasive *A. adenophora* modifies belowground soil fungi communities which is one of the mechanisms involved in the successful invasion of *A. adenophora*.

Meiners *et al.* (2017) explored the relation between soil microbial communities and plant chemistry and evaluated their impact on leaf chemical composition and allelopathic potential. They concluded that the soil microbial communities are responsible for physiological changes in plant (mainly focus on leaf tissues) of perennial plant alters allelochemical production.

The literatures related to impact on nitrogen fixing plants are very limited. Hence, the investigation to know the impact of IAPS on symbionts of nitrogen fixing plants and root nodulation would be one of the interesting issues.

CHAPTER - III

MATERIAL AND METHODS

This study was designed to explore the effect of IAPS on growth and development of nitrogen-fixing plant species. The test species were one native tree *Alnus nepalensis* and one cultivated leguminous crop (*Glycine max*). The donor species was invasive *Ageratina adenophora* and *Parthenium hysterophorus.* The study comprises pot experiment, soil analysis and microbial assay.

3.1. *Glycine max* (L.) Merr. (Soybean)

Glycine max (L.) Merr. of family Fabaceae, a subtropical plant, is one of the most important crop plants for seed protein and oil content. It is annual legume and economically the most important bean in the world, providing vegetable protein for millions of people and ingredients for hundreds of chemical products. The plant adds nitrogen to the soil through root nodules by hosting nitrogen-fixing bacteria and therefore it is considered as an important soil-enriching crop in most industrial agriculture systems. As the invasive species *Parthenium hysterophorus* and *Ageratina adenophora* have started invasion in the crop field there might be impacts of invasion in *G. max.* Indirectly, if the invasive plants are used as fertilizer, they may pose some sort of impact on nitrogen fixing ability of *G. max* either by affecting root nodules or nodule forming bacteria.

3.2. *Alnus nepalensis* D. Don.

Alnus nepalensis D. Don of the family Betulaceae is called Nepalese alder which is a fast-growing deciduous tree. Native range of *A. nepalensis* is Nepal and other south Asian countries (Orwa *et al.* 2009). The tree provides fodders, timber, poles, fibers, fuels etc. It is an excellent agroforestry tree commonly planted to restore degraded lands, to control soil erosion, landslides and floods. The tree is non-leguminous but forms a symbiotic relation with nitrogen-fixing actinomycetes of the genus *Frankia*. It forms root nodules to host the bacteria. The tree can provide a considerable quantity of nutrients through litter as well. *Ageratina adenophora* grows abundantly in the habitat of *Alnus* which might have created some sorts of impact in seedling recruitments and root nodulation.

3.3. Donor species

Ageratina adenophora (Spreng.) King & H. Rob. and *Parthenium hysterophorus* L. were the selected as donor species. Both the species are invasive annual herbs and belonging to Asteraceae family. Both have started their invasion from the southern part and have already reached to northern border crossing through Low Mountain region (Shrestha 2016). They spread from road side, pastures, fallow land, forest margin, inside forest to agro-ecosystems from eastern to western Nepal (Shrestha 2016; Thapa *et al.* 2017).

3.4. Collection of soils and seeds for experiment on *Glycine max*

For *Glycine max,* seeds were collected from Annapurna Beej Bhandar, Kalimati Kathmandu, Nepal. The seeds were screened for healthiness (selected similar size and morphologically healthy). *Glycine max* seeds were sterilized with 70% ethanol for 5 minutes and then washed several times in tap water and then seeds were spread in wet blotter paper in Petri plate for 24 hours and then sown in plastic pots. Soil sample were collected from garden of Central department of Botany.

3.5. Collection of soils and seeds for experiment on *Alnus nepalensis*

Seeds of *A. nepalensis* and soils from *A. adenophora* invaded and noninvaded sites were collected from the Champadevi Community forest, Machhegaun, Kathmandu (27°39'21''N and 85°14'47''E). This community forest is located at southwest part of Kathmandu valley. Altitude of the forest ranges from 1400 to 2300 masl. and represents a lower montane forest.

The forest has *A. nepalensis, Schima wallichii, Myrisine capitela, Castanopsis indica* etc. as the dominant native tree species. The climate of the area is hot and humid in summer and dry in winter. Annual mean temperature is approximately 18°C and average annual precipitation is about 1343 mm. Lower belt of this forest is severely invaded by *A. adenophora* which has created problems on native species seedlings recruitments (Thapa *et al.* 2017).

The noninvaded soil was collected from a homogeneous patch of *A. nepalensis* forest and invaded soil from *A. adenophora* invaded patch near *A. nepalensis* forest. At each site, a transect was made and soils were collected from five plots laying 20 m apart from each other. Composite soil was made for both invaded and noninvaded soils collected from the plots separately. The soil was transported to greenhouse of Central department of Botany and stored at room temperature until the use. Seeds of *A. nepalensis* were collected from a single tree to ensure genetic homogeneity, the seeds were air dried and finally stored in plastic bag at $5^{\circ}C$ in refrigerator.

3.6. Pot experiment

The pot experiment was conducted in the greenhouse of Central Department of Botany, Tribhuvan University, Kritipur, Kathmandu, Nepal during July to September, 2017. This experiment was developed to know the effect of *A. adenophora* and *P. hysterophorus* on growth and development *A. nepalensis* and *G. max*.

3.6.1. *Glycine max*

Seeds of *G. max* were sown in pots of size $18 \times 12\ cm^2$. Each pot contained 800 g soil which was saturated by 200 ml water before sowing seeds. In case of *G. max* pot size was large and contained 800g soil, because size of root of *G. max* were large during the treatment times. Three holes, one at bottom and 2 holes at opposite each other slightly above bottom were made in each pot. Seeds of *G. max* were sown in pot below 1 cm of soil surface. Each pot contained a single seed/plant. There were following treatments:

a. Pots treated with normal water

b. Pots treated with *A. adenophora* fresh leaf extract

c. Pots treated with *P. hysterophorus* fresh leaf extract

Every day 20 ml water and same amount of respective extracts were poured in respective pots. Each treatment had 10 replicates. In each replication only one *G. max* plant were grown, so ten replications were prepared.

3.6.2. *Alnus nepalensis*

Seeds of *A. nepalensis* were sown in pots of size $8 \times 5\ cm^2$. Each pot contained 200 g soil which was saturated by 100 ml water before sowing seeds. In this case, small pot and 200g soil only taken, because the size of *A. nepalensis* seedling is small. Three holes, one at bottom and 2 holes at opposite each other slightly above bottom were made in each pot. The seeds of *A. nepalensis* were soaked in tap water for 24 hrs. After that, 10 soaked seeds were sown in pots below 1 cm of surface soil treated with 20 ml water. After 25 days of sowing, the seedlings were removed by allowing only four *Alnus* seedlings at each pot. The pots were treated as follows:

a. Non-invaded soil (NIS)

b. Non-invaded soil + *A. adenophora* litter (NIS + LI)

c. Non-invaded soil + *A. adenophora* fresh leaf extract (NIS + FLE)

d. Invaded soil (IS)

e. Invaded soil + *A. adenophora* litter (IS + LI)

f. Invaded soil + *A. adenophora* fresh leaf extract (IS + FLE)

Each treatment had 5 replications. Twenty ml of water was added in alternate day in control (uninvaded soil and invaded soil) and litter treated pots. Same amount of fresh leaf extract was added in same manner in pots with treatment of uninvaded and invaded soil + *A. adenophora* fresh leaf extract. Crushed leaf litter of *A. adenophora* (2 g) was placed on the soil surface of litter treated pot.

The pots of both *G. max* and *A. nepalensis* were frequently randomized. The temperature of the greenhouse ranged from 25-44.4^{o}C and moisture ranged from 55 to 87%.

3.7. Preparation of fresh leaf extract

Fresh leaves of both invasive species were collected from surrounding of Central Department of Botany, Tribhuvan University, Kathmandu, Nepal. Fresh leaves (15 g) were soaked in 100 ml water for 24 hours to prepare leaf extract. The extract was directly used and the extract prepared at one time was stored at 5°C in refrigerator for 2 days.

3.8. Measuring parameters

Glycine max was harvested after 2 months of sowing data and *A. nepalensis* after 3 months. Then, shoot length, root length, leaves number, nodule number, internode number, branch and leaf number, dry shoot biomass and dry root biomass were measured.

3.9. Soil analysis

Soil samples were collected from pots treated to analyze total soil nitrogen and soil pH. Total soil nitrogen content of treated soil was measured by Micro-Kjeldhal method (Jackson 1967) and pH by Fischer's digital pH meter in 1:2 ratio of soil water mixture. The soil samples were tested in Nepal Bureau of Standard and Meteorology Lab, Kathmandu, Nepal.

3.10. Mycorrhizal analysis

Mycorrhizal study was done only for *Alnus nepalensis*. First of all, 2 cm long root of *Alnus* were cut and placed in sterile water. Then, root was dipped 10% KOH solution for 12 h and then boiled until the root is clear. Cleared roots were stained with ink solution about 5 min, washed the root with distilled water and finally the mycorrhizae were observed under compound microscope (Brundrett *et al.*1996). Randomly selected 50 root pieces from one treatment (10 root pieces from each pot treated) were observed for presence or absence of mycorrhiza. The mycorrhizae were dark blue stained. Percentage of mycorrhizal colonization was calculated by using formula:

$$\%\ \text{mycorrhizal colonization} = \frac{\text{No. of root pieces having mycorrhizae} \times 100}{\text{Total No. of root pieces observed}}$$

3.11. Statistical analysis

All the measuring parameters such as root and shoot growth, root and shoot biomass, branch; leaf and nodule number, soil pH, total soil nitrogen and mycorrhizae association percentage were compared using One-way ANOVA and t- test in SPSS (version 20).

CHAPTER - IV

RESULTS

4.1. Effect of *Ageratina adenophora* on *Alnus nepalensis*

4.1.1. **Shoot and root length**

The results showed significant reduction in the shoot and root length by non- invaded soil + fresh leaf extract (NIS + FLE) ($P < 0.01$). Interestingly, both the shoot and root length were not inhibited significantly by other treatments such as the invaded and non-invaded soil (IS and NIS), invaded soil + litter (IS + LI), non-invaded soil + litter (NIS + LI) and invaded soil + fresh leaf extract (IS + FLE) (Fig. 1 and 2, Annex I – Table 1).

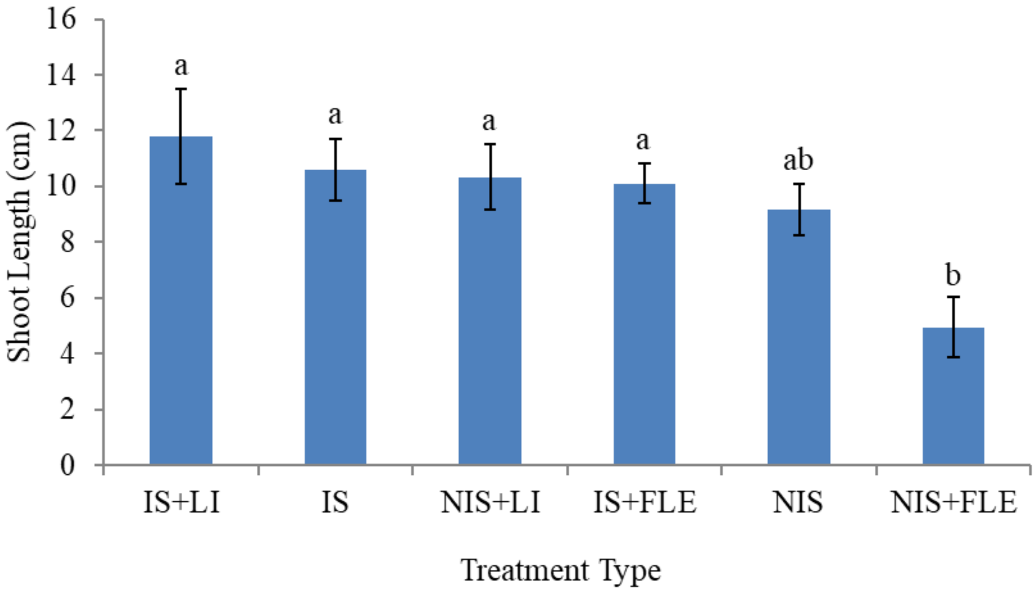

Fig. 1 Shoot length of *A. nepalensis* under different treatments [invaded soil (IS), non-invaded soil (NIS), invaded soil + litter (IS + LI), non-invaded soil + litter (NIS + LI), invaded soil + fresh leaf extract (IS + FLE) and non-invaded soil + fresh leaf extract (NIS + FLE)]. The letters above error bar shows significant differences among the treatments.

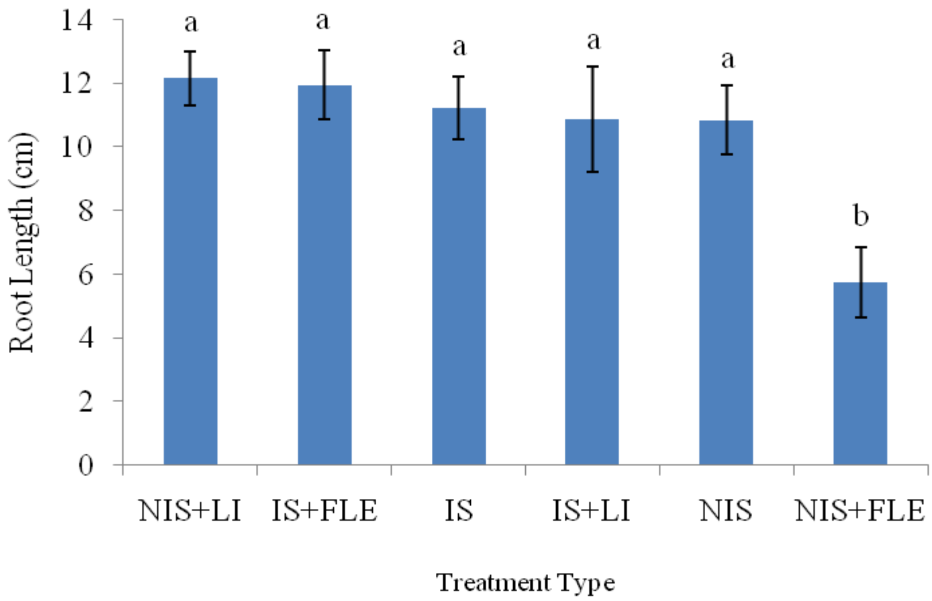

Fig. 2 Root length of *A. nepalensis* under different treatments [invaded soil (IS), non-invaded soil (NIS), invaded soil + litter (IS + LI), non-invaded soil + litter (NIS + LI), invaded soil + fresh leaf extract (IS + FLE) and non-invaded soil + fresh leaf extract (NIS + FLE)]. The letters above error bar shows significant differences among the treatments.

4.1.2. Leaf number

Similar to the effect on root and shoot length there were significant reduction in leaf number by non-invaded soil + fresh leaf extract (NIS + FLE) ($P < 0.01$) but invaded soil + fresh leaf extract (IS + FLE) did not show any inhibitory or stimulatory effect. Similarly, other treatments with invaded or noninvaded soils (IS and NIS), invaded + litter (IS + LI) and non-invaded soil + litter (NIS + LI) also did not reduce the leaf number (Fig. 3, Annex I-Table 1).

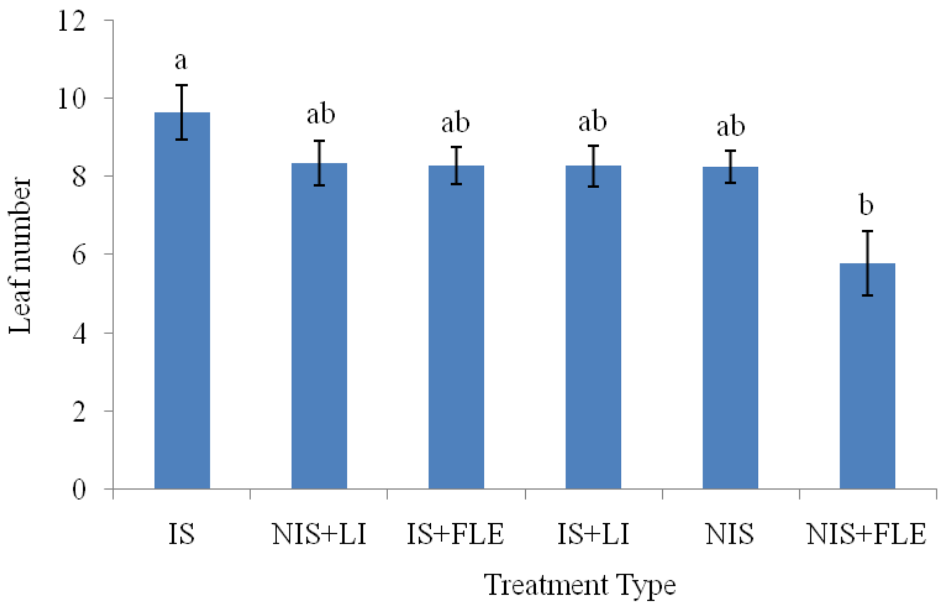

Fig. 3 Leaf number of *A. nepalensis* under different treatment [invaded soil (IS), non-invaded soil (NIS), invaded soil + litter (IS + LI), non-invaded soil + litter (NIS + LI), invaded soil + fresh leaf extract (IS + FLE) and non-invaded soil + fresh leaf extract (NIS + FLE)]. The letters above error bar shows significant differences among the treatments.

4.1.3. Shoot and root biomass

Similar to the shoot and root length the non-invaded soil + fresh leaf extract (NIS + FLE) inhibited the shoot biomass ($P = 0.012$, Fig. 4) while there was no inhibition in root biomass ($P = 0.081$, Fig. 5). Invaded soil and non-invaded soils (IS and NIS), invaded soil + litter (IS + LI), non-invaded soil + litter (NIS + LI), invaded soil + fresh leaf extract (IS + FLE) did not show inhibition to both shoot and root biomass (Fig. 4 and 5, Annex I- Table 1).

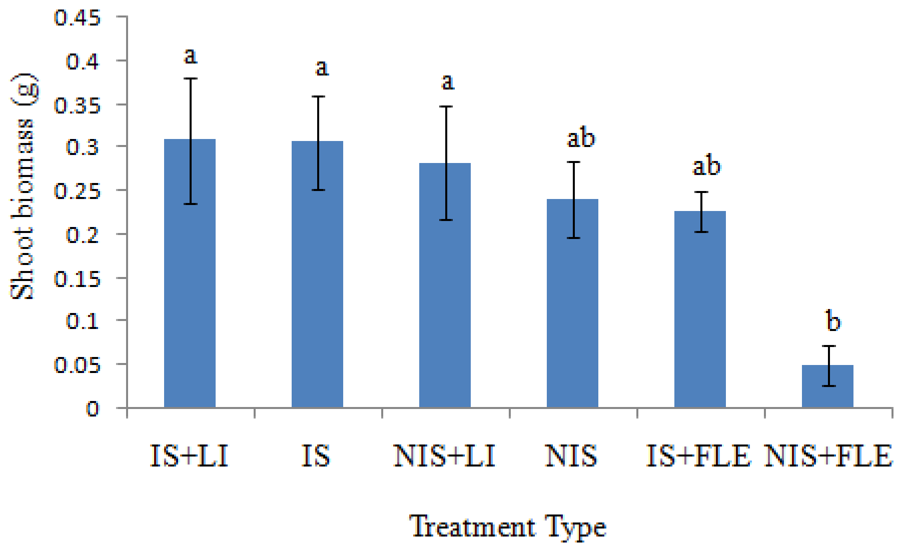

Fig. 4 Shoot biomass *A. nepalensis* under different treatments [invaded soil (SI), non-invaded soil (NIS), invaded soil + litter (IS + LI), non-invaded soil + litter (NIS + LI), invaded soil + fresh leaf extract (IS + FLE) and non-invaded soil + fresh leaf extract (NIS + FLE)]. The letters above error bar shows significant differences among the treatments.

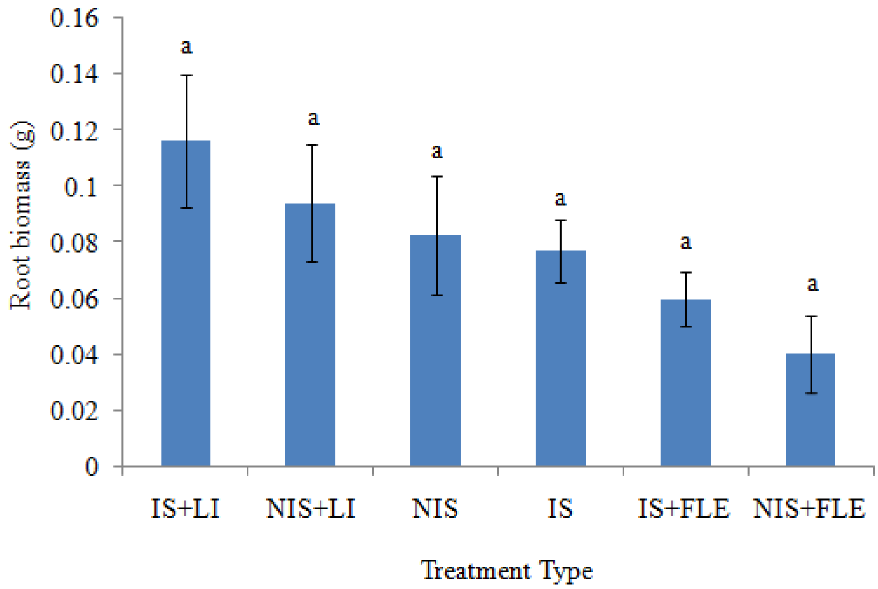

Fig. 5 Root biomass of *A. nepalensis* under different treatments [invaded soil (IS), non-invaded soil (NIS), invaded soil + litter (IS + LI), non-invaded soil + litter (NIS + LI), invaded soil + fresh leaf extract (IS + FLE) and non-invaded soil + fresh leaf extract (NIS + FLE)]. The letters above error bar shows significant differences among the treatments.

4.1.4. Root nodule number

The interesting result was obtained in root nodule in *A. nepalensis* i.e. the visible root nodules were only present in the invaded soils (IS) but not in non-invaded soils (NIS). The nodules found under the treatments (invaded soil and litter or leaf extract with invaded soil) were not significantly different (P = 0.410, Fig. 6, Annex I- Table 1).

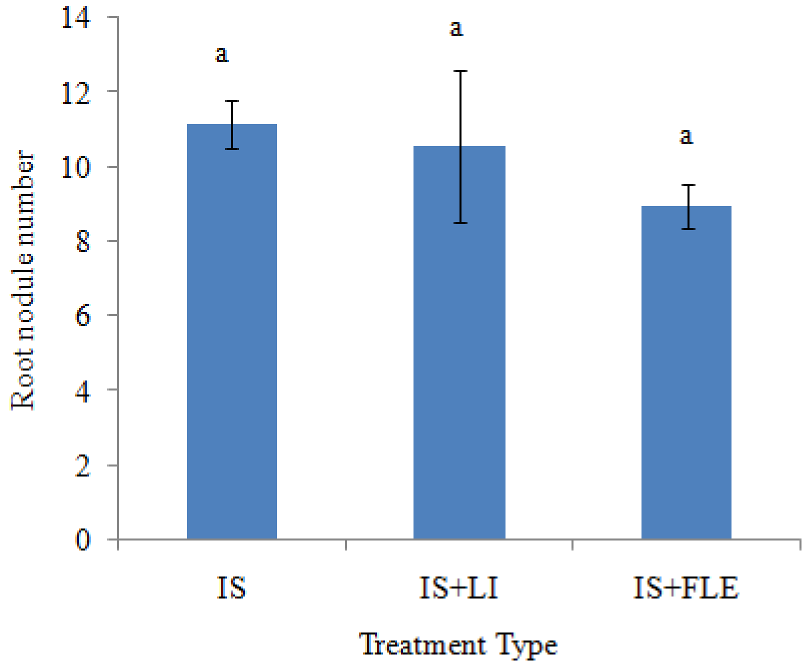

Fig. 6 Root nodule number of *A. nepalensis* under different treatments [invaded soil (IS), invaded soil + litter (IS + LI), invaded soil + fresh leaf extract (IS + FLE)]. The letters above error bar shows significant differences among the treatments.

4.2. Effect of *A. adenophora* on soil pH and nitrogen content

4.2.1. Soil pH

The differences in pH of soil among various treatments were significantly different (P <0.001). The pH was comparatively higher in the invaded soils and invaded soil + either fresh leaf extract or litter than the non-invaded soils and non-invaded soil + fresh leaf extract or litter (Fig. 7, Annex I- Table 1).

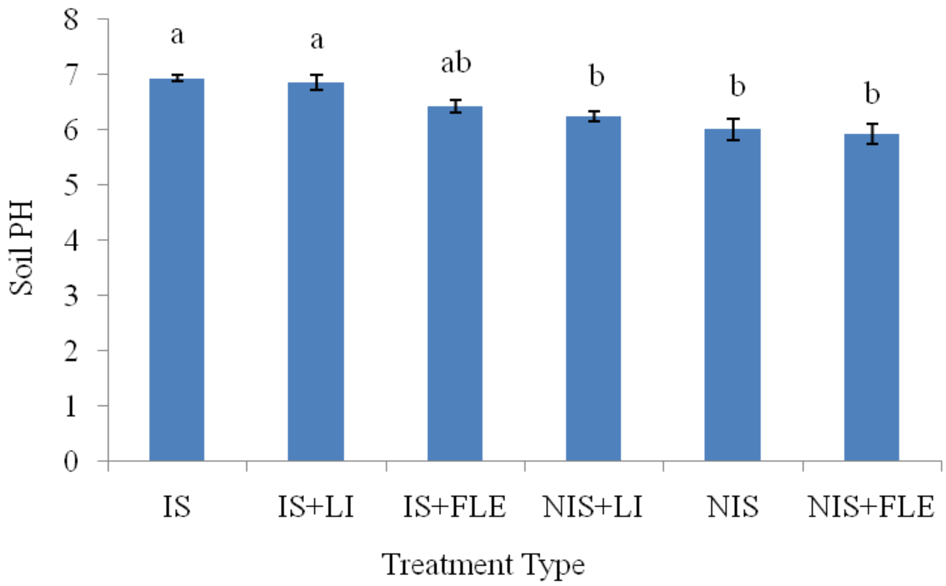

Fig. 7 Soil pH in *Alnus nepalensis* grown soils [invaded soil (IS), non-invaded soil (NIS), invaded soil + litter (IS + LI), non-invaded soil + litter (NIS + LI), invaded soil + fresh leaf extract (IS + FLE) and non-invaded soil + fresh leaf extract (NIS + FLE)]. The letters above error bar shows significant differences among the treatments.

4.2.2. Nitrogen content

Just reverse to the result of pH, content of soil nitrogen was found high in the non-invaded soils (NIS) and non-invaded soils with either litter or fresh leaf extract (NIS + LI and NIS + FLE) (Fig. 8, P = 0.001, Annex I- Table 1.). Among the invaded soils, the invaded soil + fresh leaf extract (IS + FLE) had reduced the nitrogen content greatly comparing to the invaded soil (IS) and invaded soil + litter (IS + LI) (Fig. 8).

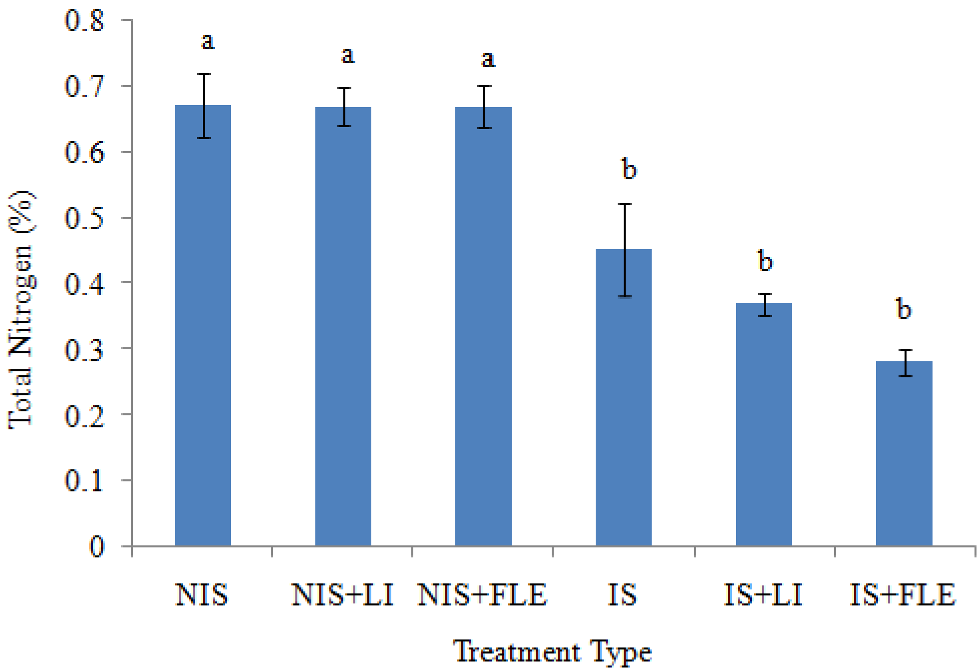

Fig. 8 Total nitrogen content in *Alnus nepalensis* grown soils [invaded soil (IS), non-invaded soil (NIS), invaded soil + litter (IS + LI), non-invaded soil + litter (NIS + LI), invaded soil + fresh leaf extract (IS + FLE) and non-invaded soil + fresh leaf extract (NIS + FLE)]. The letters above error bar shows significant differences among the treatments.

4.3. Effect of *A. adenophora* on mycorrhizal association in *A. nepalensis*

Mycorrhizal association in the non-invaded soil + litter (NIS + LI) was the highest and in the invaded soil + litter (IS + LI) was the lowest (Fig. 10, P = 0.013). The invaded soil (IS), non-invaded soil (NIS), and fresh leaf extract with invaded and non-invaded soils (IS + FLE and NIS + FLE) did not change the association of mycorrhizae in *A. nepalensis* (Fig. 10, Annex I-Table 1).

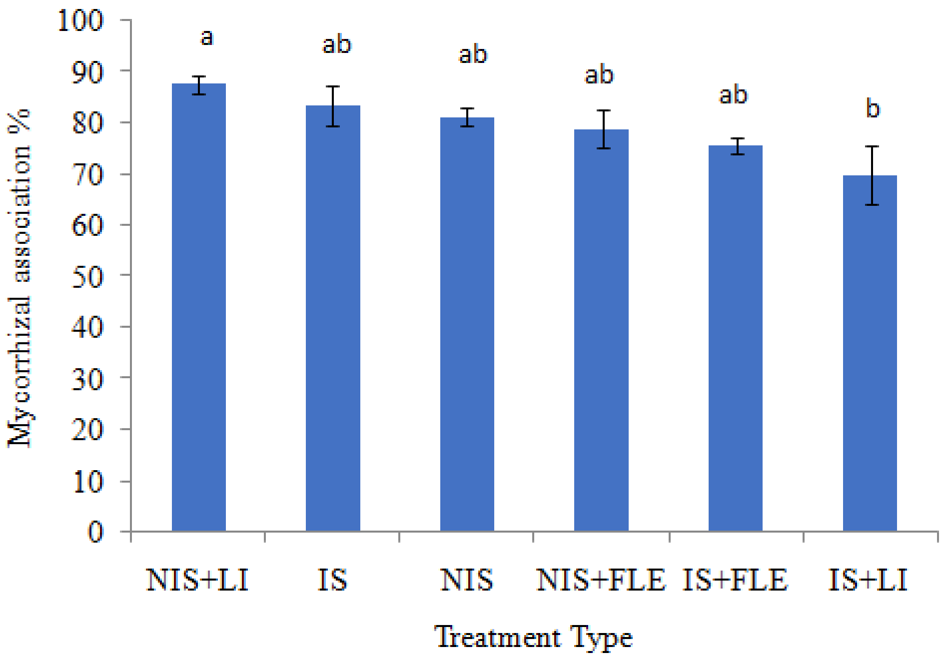

Fig. 9 Mycorrhizal association in *A. nepalensis* in different treatments [invaded soil (IS), non-invaded soil (NIS), invaded soil + litter (IS + LI), non-invaded soil + litter (NIS + LI), invaded soil + fresh leaf extract (IS + FLE) and non-invaded soil + fresh leaf extract (NIS + FLE)]. The letters above error bar shows significant differences among the treatments.

4.4. Effect of *Ageratina adenophora* and *Parthenium hysterophorus* on *Glycine max*

4.4.1. Shoot and root length

The shoot and root length of *G. max* was not affected by both *P. hysterophorus* and *A. adenophora* leaf extracts (P = 0.266, Fig. 10 and 11). Although, the shoot and root were comparatively longer in control than the plants that were grown in soils treated with invasive extracts (Fig. 10 and 11, Annex I-Table 2).

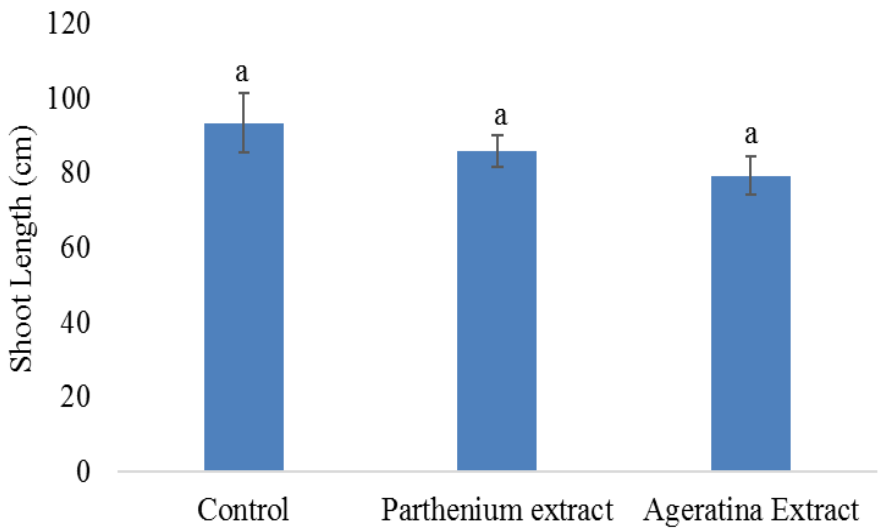

Fig. 10 Shoot length of *G. max* grown in control and invasive plant's leaf extract. The letters above error bar shows significant differences among the treatments.

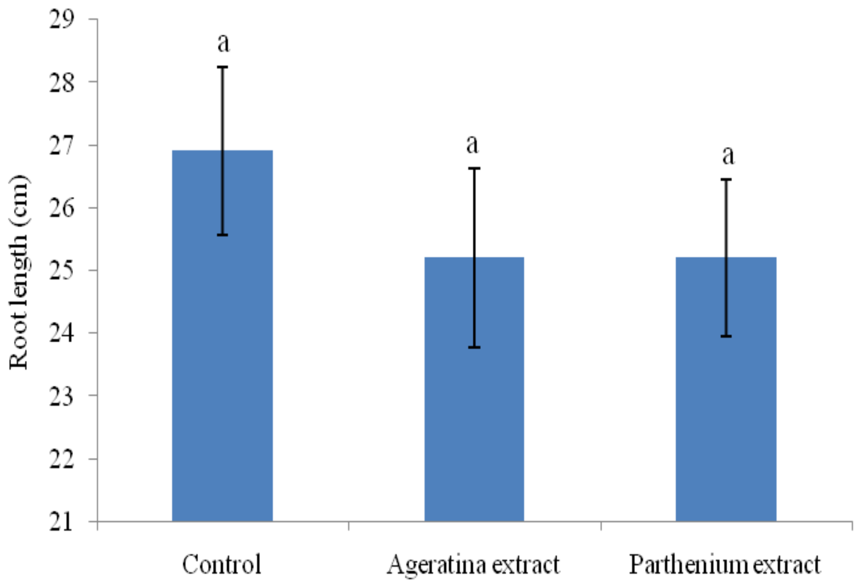

Fig. 11 Root length of *G. max* grown in control and invasive plant's leaf extract. The letters above error bar shows significant differences among the treatments.

4.4.2. Number of leaves, internodes and branches

Both the *A. adenophora* and *P. hysterophorus* leaf extracts reduced the number of leaves in *G. max* significantly (P = 0.016, Fig. 12) but the number of branches and internodes was not affected by the extracts significantly (P = 0.839 and 0.248 respectively) (Fig. 12 and 13, Annex I-Table 2).

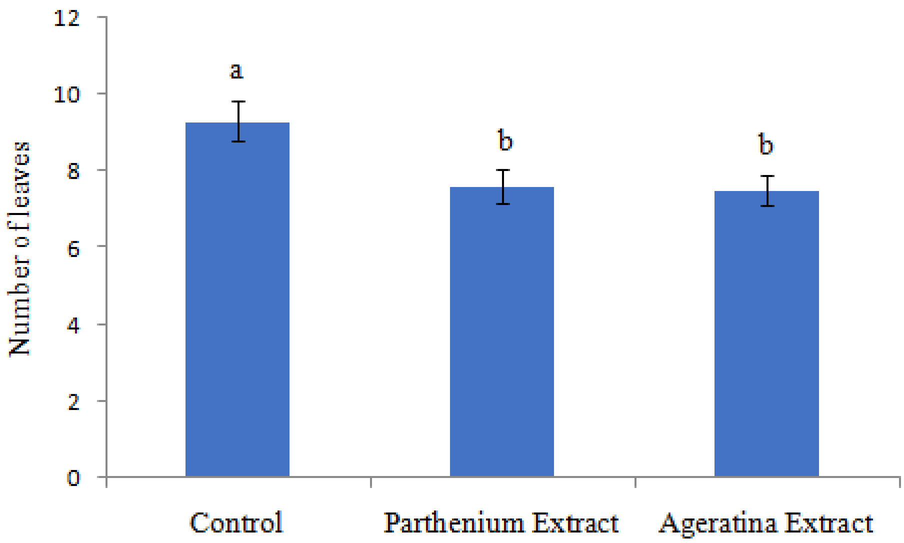

Fig. 12 Number of leaves of *G. max* grown in control and invasive plant's leaf extract. The letters above error bar shows significant differences among the treatments.

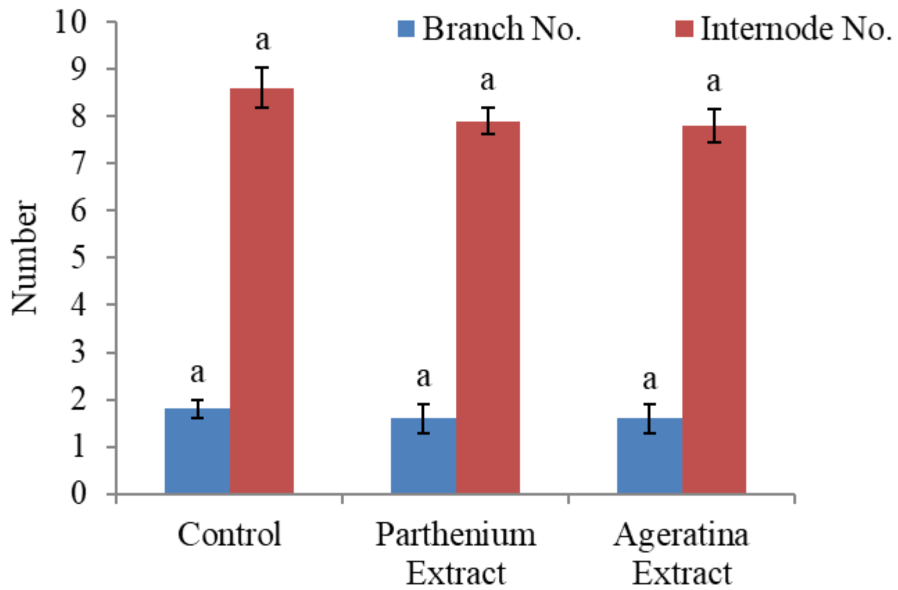

Fig. 13 Branch number and Internodes number of *G. max* grown in control and invasive plant's leaf extract. The letters above error bar shows significant differences among the treatments.

4.4.3. Shoot and root biomass

The shoot biomass of *G. max* in the control was high than that was treated with the invasive species leaf extracts. There was no difference in the biomass between *P. hysterophorus* and *A. adenophora* extracts. The differences between control and extract treatments was significant (P = 0.01, Fig. 14, Annex I- Table 2). In case of the root biomass, there was no differences among control and extract of both invasive species (P = 0.117, Fig. 15, Annex I-Table 2).

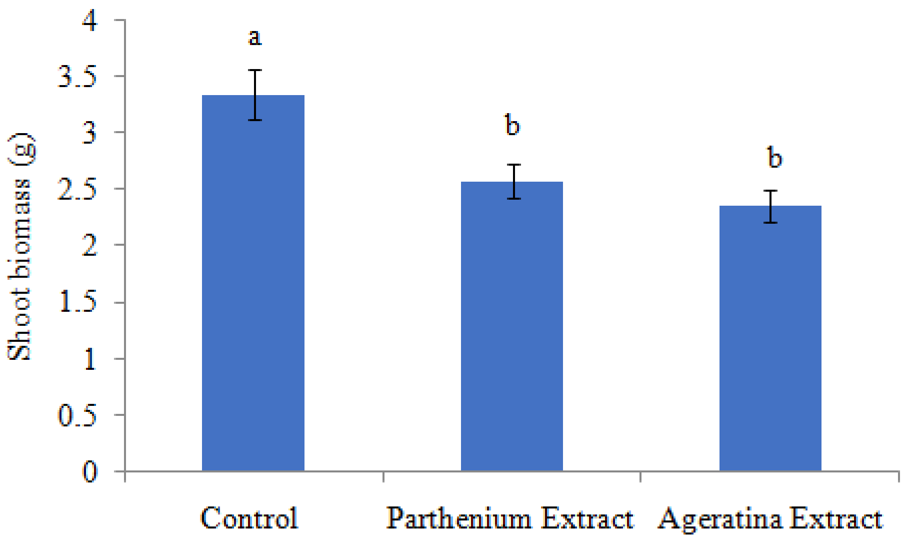

Fig. 14 Shoot biomass of *G. max* grown in control and invasive plant's leaf extract. The letters above error bar shows significant differences among the treatments.

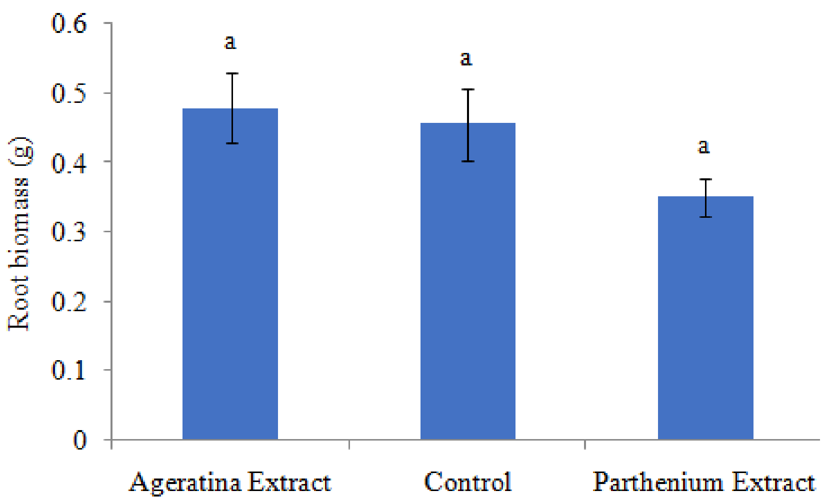

Fig. 15 Root biomass of *G. max* grown in control and invasive plant's leaf extract. The letters above error bar shows significant differences among the treatments.

4.4.4. Nodule number

Number of nodules were significantly higher in *A. adenophora* fresh leaf extract treatment than control (P = 0.05) but the nodules were absent in *P. hysterophorus* fresh leaf extract treated plants (Fig. 16, Annex I-Table 3).

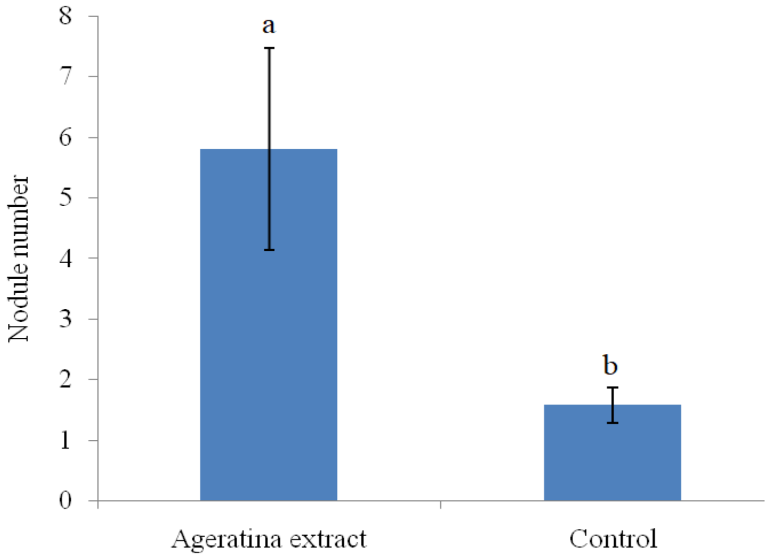

Fig. 16 Number of root nodules of *G. max* grown in control and invasive plant's leaf extract. The letters above error bar shows significant differences among the treatments.

4.4.5. Soil pH and nitrogen content

There was no significant change in soil pH by the addition of leaf extracts from *P. hysterophorus* and *A. adenophora* (P = 0.512, Fig 17, Annex I-Table 2). Similar was the case in soil nitrogen content although *A. adenophora* extract had increased the content slightly comparing to the control and extract of *P. hysterophorus* (P = 0.110, Fig. 18, Annex I-Table 2).

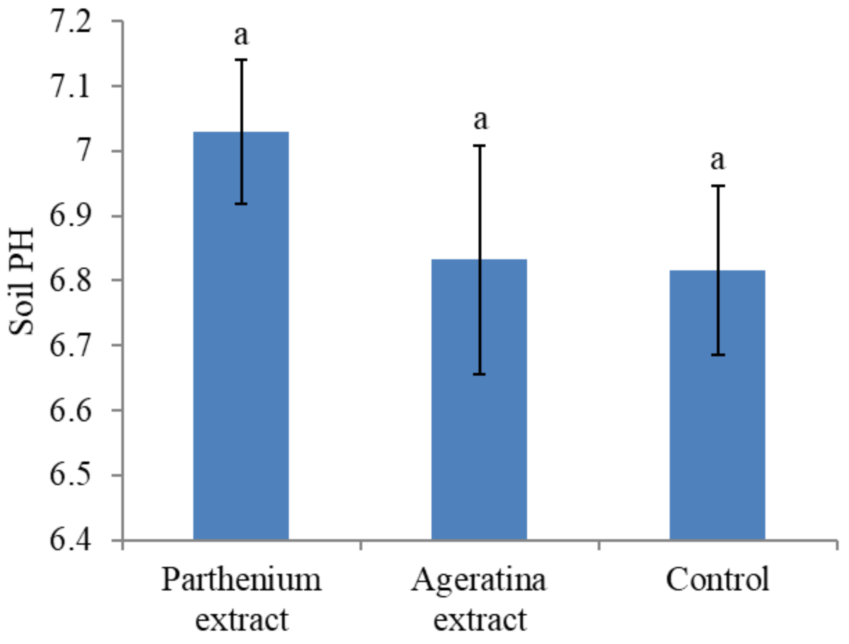

Fig. 17 pH of soil in control and soil treated by invasive plant's leaf extract. The letters above error bar shows significant differences among the treatments.

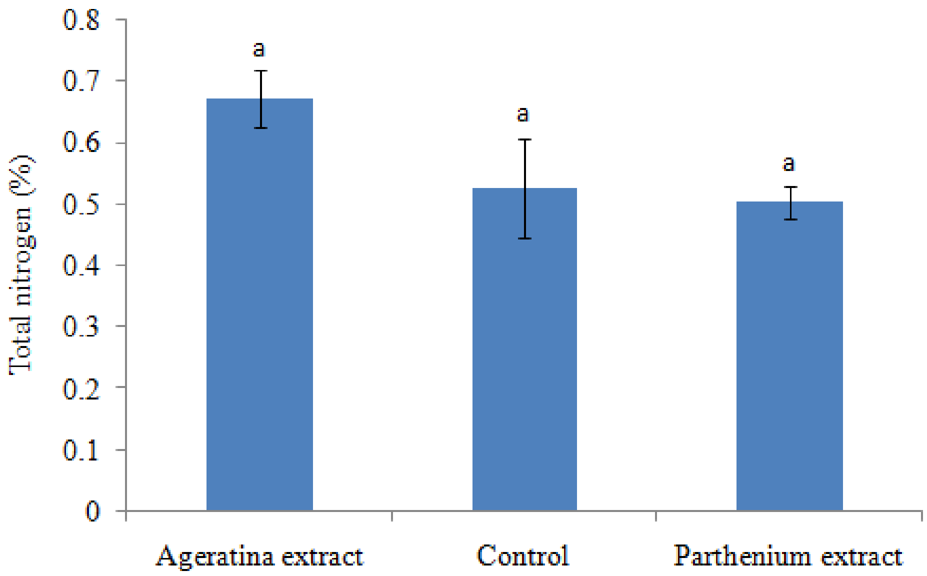

Fig. 18 Total nitrogen of soil in control and soil treated by invasive plant's leaf extract. The letters above error bar shows significant differences among the treatments.

CHAPTER – V

DISCUSSION

5.1. *Ageratina adennophora* hinders growth and development of *Alnus nepalensis*

The shoot and root length, leaves number and shoot biomass was reduced by NIS + FLE only, other treatment did not show any differences. It was interesting to note that addition of fresh leaf extract in invaded soil showed no effect on these parameters. It indicates that, there might be some interesting interaction among soil type, Fresh leaf extract and plant growth response.

Basically, there might be some changes brought by addition of Fresh leaf extract in Noninvaded soil. The question is that why fresh leaf extract did not reduce the growth parameters? As the result showed that, there is no impact of Invaded soil on growth parameters. It means seedling may response differently, when they are transplanted to different soil type.

Analysis of PH shows that the reduction in growing parameters is not due to pH because the pH is lower not only in NIS + LI but also pH is significantly lower in NIS and NIS + FLE (Fig. 7). It is obvious that the pH in noninvaded soil is lower than the invaded soil (Fig. 7).

While comparing nitrogen content in the soil treatment, it was found that the invaded soil can be reduced the nitrogen content (Fig. 8). If we expect that there is role of nitrogen in the *Alnus* seedling growth, it will be not true logic because the nitrogen content is not lower in NIS + FLE.

From this study it was not clear that, what factors are responsible for reducing *Alnus* seedling parameter only in NIS+FLE. Further studies to evaluate other nutrients which play role in seedling growth can be suggested in such condition. In the invaded soil, there might be some content of allelochemicals present already and hence, after seedling transplantation the seedling got chance to adapt more allelochemicals since the initial day of transplantation.

In case of noninvaded soil, the seedling gradually faced to the higher amount of FLE during the course of experiment. As all the pots were placed in same environment and the pots were randomized regularly other environmental factors cannot be consider as influencing factors on growth and development.

Various allelopathic chemicals such as quinic acid derivative, 5-O-trans-o-coumaroylquinic acid methyl ester, chlorogenic acid methyl ester, macranthoin F and macranthoin G, are found in the aerial parts of *Ageratina adenophora* (Zhang *et al.* 2013). These chemicals might have reduced growth and development of tested native seedlings. The results are similar to the findings of Zhong *et al.* (2007), Zhang et al. (2008) and Thapa *et al.* (2017).

5.2. Invaded soil enhances nodulation in *Alnus nepalensis*

The result of study showed that, nodules were not developed in noninvaded soil until the time of harvesting the seedling but the nodules appeared in invaded soil (Fig. 6). Comparing nodules with nitrogen content the result is somewhat interesting, that is nitrogen content is reduced by invaded soil and reduction increases while adding litter and fresh leaf extract on the invaded soil (Fig. 8). It clearly shows that, reduction in nitrogen content stimulates formation of root nodules. It might also be due to effect of allelochemicals on root nodule bacteria of *Alnus* i.e. *Frankia.* Either the allelochemicals are responsible to stimulate the association between root cells and *Frankia*, or the *Frankia* might have developed root nodules faster to be protected from the allelochemicals.

Root nodules were only appeared in invaded soil but totally absent in non-invaded soil. This was might be due to present of allelochemicals in invaded soil. So, nitrogen fixing bacteria (symbiont) try to protect from these allelochemicals and more then penetrated as fast as possible. Therefore, *Alnus* grown in invaded soil had root nodules. Another reason might be due to less nitrogen content in invaded soil, plant have capacity to fulfill nitrogen content in plant by help of symbiont in root. Thus, formed root nodules.

Studies on the effect of *A. adenophora* on symbiotic bacteria in the soil are rarely done. Xu *et al.* (2012) have concluded that the number of nitrogen-fixing bacteria and species diversity were significantly greater in *A. adenophora* invaded sites than the noninvaded soils. This might be the reason behind increasing the root nodules in *A. nepalensis.*

The associations of mycorrhizae also have no impact on growth and development of *Alnus* seedling because the percentage mycorrhizal association is significantly lower only in IS + LI. Analysis of mycorrhiza clearly indicates that interaction of IS + LI reduces the percentage colonization significantly. Therefore, it is one of the important expect be consider.

Mycorrhizae are responsible for the supply of water and nutrients to the plants. They exchange various substances with the shoots. It supports aeration and plant biological activities (Varma 1998). Stinton *et al.* (2006) have studied the invasive plant *Alliaria petiolata* suppressed native plant growth by disrupting the mycorrhizal association. Mycorrhizal were positively associated with field water holding capacity, pH and total nitrogen content but negatively associated with organic matters (Becerra *et al.* 2005). This result shows that non-invaded soil with litter might have high water holding capacity due to fine soil and had total nitrogen content also high but pH was acidic.

5.3. Growth, development and nodulation in *Glycine max* under treatment of *Parthenium hysterophorus* and *Ageratina adenophora*

There were significant differences in leaf number (Fig. 13) and shoot biomass (Fig. 15) of *G. max* plants. This might be due to presence of allelochemicals in the fresh leaf extract of both invasive alien plants. Thiebaut *et al.* (2018) have studied fresh leaf extract of invasive plant *Ludwigia hexapetals* reducing the leaf area of *Myriophyllum aquaticum*. The shoot biomass might have reduced due to reduction in the leaf number of *Glycine max*. Batish *et al.* (2002) concluded that the presence of phenolics in *Parthenium* residues and their interference with soil chemistry upon release may reduce the growth and development of vegetable crops like radish and chickpea. The findings of this study also support the findings of Pandey (1994), Tefera (2002), Wakjira (2009) and Shabbir and Jayaid (2010).

Root nodules were significantly present on *A. adenophora* fresh leaf extract treated *G. max* but the nodules were absent on treatment of *P. hysterophorus* fresh leaf extract.

This indicates that the *P. hysterophorus* might have interfered the mechanism of root nodules. It is interesting to note that the *A. adenophora* has reduced nitrogen content in soil in *A. nepalensis* pots but the nitrogen content was not reduced in the pots of *G. max.* It was expected that the reduced nitrogen in the soil stimulated root nodulation in *A. nepalensis* for accumulation of nitrogen. Moreover, it can be said that *A. adenophora* played positive role in association of *Frankia* with *A. nepalensis* to form root nodules.

In case of *G. max* there was no reduction in nitrogen content in the soil by *A. adenophora* (Fig. 19) even though the number of root nodules increased. Here, there might be no relation of soil nitrogen to form root nodules in *G. max.* It might be due to different type of interaction among *G. max, A. adenophora* and nodule forming soil bacteria *Rhizobia* than the interactions happen in *A. nepalensis.* It can be expected that the *Rhizobia* might have affected by the allelochemicals present in fresh leaf extract of *A. adenophora* and they migrated into the roots faster and large in number than the untreated soil. Kanchan and Jayachandra (1981) had concluded that the root or leaf extract of *Parthenium hysterophorus* inhibits the growth of *Rhizobium phaseoli* and *Azotobacter vinelandii*. They also concluded that the inhibition was caused by parthenin, caffeic acid, and anisic acid, the important inhibitors present in the weed. Overall, there should be a complex and interesting interaction between nodule forming bacterial activities and allelochemicals of invasive species which ultimately affects on plant growth and development or in soil feedback mechanisms.

CHAPTER - VI

CONCLUSION AND RECOMMENDATION

6.1. Conclusion

From the results it can be concluded that seedling growth of native tree *(A. nepalensis*) is directly or indirectly affected by *A. adenophora*. Addition of *A. adenophora* fresh leaf extract is harmful to *A. nepalensis* root-shoot growth and development. Positive or negative interaction of fresh leaf extract depends on the soil types i.e. non-invaded soil or invaded soil. Similarly, response of growing seedlings to the allelochemicals also depends on the soil type. Invaded soil can reduce soil nitrogen and hence the root nodules in *A. nepalensis* are stimulated. It might be also due to effect of allelochemicals on nodule forming bacteria the *Frankia.*

Both invasive species *A. adenophora* and *P. hysterophorus* also affect on growth and development of *G. max*. Infestation of these invasive species in *G. max* field reduces number of leaves and biomass which may lead to loss of production. Beneficial interaction is that the *A. adenophora* stimulates nodule formation in *G. max.* It might be due to direct or indirect effect of allelochemicals to nitrogen fixing bacteria *Rhizobia.* Overall, the interaction among products of invasive alien species, soil microorganisms and soil nutrients depend on plant types.

6.2. Recommendation

Ageratina and *Parthenium* should be controlled for well regeneration of *Alnus* seedlings and *G. max* crops. Other parameters of the soil are also need to be analyzed to know soil nutrients dynamic affected by *Ageratina* and *Parthenium* which is yet contradictory. Further studies are recommended for confirming the results and understanding plant-soil interactions.

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Annex I

Table 1: ANOVA table on various *A. nepalensis* growth parameters

Parameters	Sum of Squares	Df	Mean Square	F	P value
Shoot Length	141.562	5	28.312	4.243	0.007
Root length	141.715	5	28.343	4.243	0.007
Leaf number	39.705	5	7.941	4.431	0.005
Shoot biomass	0.235	5	0.047	3.711	0.012
Root biomass	0.017	5	0.003	2.256	0.081
pH	4.500	5	0.900	9.613	<0.001
Nitrogen	0.798	5	0.160	19.367	<0.001
Mycorrhizal association	1250.551	5	250.110	3.652	0.010
Root nodules	14.284	2	7.142	0.953	0.410

Table 2: One-way ANOVA on *G. max* seedlings growth parameters

Measuring Parameters	Sum of Squares	Df	Mean Square	F	P value
Shoot length	1002.62	2	501.31	1.39	0.27
Root length	19.27	2	9.63	0.54	0.59
Leaf number	20.47	2	10.23	4.85	0.02
Branch number	0.27	2	0.13	0.17	0.84
Shoot biomass	5.31	2	2.66	8.57	0.01
Root biomass	0.142	2	0.71	3.98	0.03
PH	0.142	2	0.071	0.708	0.512
Nitrogen	0.083	2	0.041	2.666	0.110

Table 3: T-test on the root nodule present of *G. max*

	t	Df	P value	Mean Difference
Nodule Number	3.702	16	0.002	4.05882

Photo Plates

Alnus nepalensis seedling in nature

Soil collection

Ageratina adenophora

Alnus nepalensis in Champadevi, Kathmandu)

Alnus nepalensis seedlings grown in pots

Alnus root nodules

Oral presentation in 4th Graduate Conference on Environment and Sustainable Development, Kathmandu (2018) and Poster presentation in the 2nd International conference on Mountain in the Changing World, Kathmandu (2018).

Glycine max grown in pots

Working in Laboratory